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PATENT SPECIFICATION

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(72) Inventors KENNETH VICTOR SLEE
TIMOTHY CHARLES APPLETON

(19)

(54) IMPROVEMENTS RELATING TO CRYOSTATS

(71) We, SOUTH LONDON ELECTRICAL EQUIPMENT COMPANY LIMITED, of Lanier Works, Hither Green Lane, London, SE13 6QD, a Company organised under the laws of Great Britain, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to an improved cryostat assembly for a microtome to be used for cutting thin sections of biological tissue at low temperatures.

A broadening field of interest is being developed in the cutting of thin fresh frozen sections of tissue suitable for use with electron microscope work. To satisfactorily deliver such minute sections, an ultra-microtome cryostat is used and the following are desirable features which, though not absolutely necessary, do nevertheless greatly contribute to the delivery from the microtome of much improved specimens down to 250 Angstroms in thickness, the equivalent of which would otherwise be virtually unobtainable.

1) Precise control

2) Vibration free (this is necessary to achieve (1) above)

3) Advantageous environment (non-oxidizing dry atmosphere)

4) Absolutely silent (thus avoiding distraction liable to upset the concentration of the operator)

5) High degree of reliability with simple maintainance.

According to the present invention there is provided a cryostat assembly comprising a thermally insulated cryostat chamber and a thermally insulated container in non-closable free-flow fluid communication therewith, the container being adapted to contain a liquefied non-oxidizing gas for cooling the chamber to a predetermined temperature, the supply of liquified gas through the fluid communication to the chamber being controlled by control means including an electric heating means positioned inside the thermally insulated container, the control means controlling the supply of current to said electric heating means so as to supply heat to the liquified gas in said container to thereby vapourise controllable quantities of said liquified gas to pressurise the container, the pressure so generated forcing a controlled quantity of the liquified gas through the fluid communication into said chamber, the arrangement being such that the said controlled quantity of gas evaporates in said chamber to cool the chamber.

Preferably a standby mechanical refrigeration system is provided to maintain a standby temperature higher than said predetermined temperature in the cryostat.

Preferably also a vacuum probe is provided to manipulate the cut sections of material cut by said microtome.

Preferably also an insulated light-tight compartment is provided within said chamber for storing and freeze drying of the cut sections of material.

Preferably also means are provided for controlling the temperature of the microtome knife independently of the temperature of the specimen being cut or of the prevailing temperature inside said chamber.

A preferred embodiment of the present invention will now be described with deference to the accompanying drawings in which:—

Figure 1 is a sectional side view of a cryostat

Figure 2 is an enlarged detailed side view of part of the apparatus shown in Figure 1.

An ultra-microtome 1 of any known type that is a microtome for cutting extremely

thin sections down to 250 Angstroms thickness, is located in a thermally insulated refrigeration chamber 2 of a cryostat 3 which is normally maintained at a standby temperature of approximately $-40°C$ by means of the evaporator 4 of a refrigeration unit 5.

When the equipment is required for operational purposes, however, the refrigeration unit 5 is cut out and an entirely different refrigeration system is brought into service.

This alternative arrangement relies on the use of a low temperature non-oxidizing gas such as liquid nitrogen which is injected into the chamber 2 by means of a control system 6 which in effect controls the electric current passing through a small electric heater 7 located in a Dewar flask or storage vessel 8 containing liquid nitrogen. The heater 7 causes some of the liquid gas to vapourise which pressurises the flask 8 and thus forces some of the liquid nitrogen out of the flask 8 and causes it to drip via a tube 9 into the ultramicrotome chamber 2 in small globules. These globules "run around" the base of the chamber 2 until they evaporate themselves off, thus cooling the interior of the chamber down to $-100°C$ if desired.

Thus no mechanically moving parts, such as valve gear, are involved and the temperature of the chamber 2 is regulated purely by regulating the amount of liquid nitrogen fed into it and this is done by controlling the electric current passing through the heater 7.

The liquid nitrogen advantageously provides a non-oxidizing atmosphere which is an ideal environment for the minute sections to be cut by the microtome 1 as oxidation of the tissue is thereby greatly reduced.

As the sections are cut by the ultramicrotome 1 they form a ribbon which is lifted away from the knife 10 of the microtome 1 and held suspended in the ambient nitrogen atmosphere by means of a vacuum probe 11 mounted on a micro manipulator 12 which has three manually operable controls for controlling the movement of the probe in three mutually perpendicular planes, the controls all being mounted externally of the chamber 2. This unit 12 is also used to transfer the cut sections on to a suitably positioned electron microscope grid 13 2mm in diameter, which in turn may be transferred to an independently temperature controlled light-tight cell 14 for storage or freeze drying.

This separate refrigerated cell 14 is incorporated inside the main chamber 2 and includes an independently controllable temperature control system 14*a* so that exposure of specimens to damaging temperatures or environments is also avoided.

The inherent simplicity of the whole arrangement is self evident.

The temperature of the glass or diamond knife 10 can be maintained at a higher level than the temperature of the specimen being cut or of the prevailing temperature inside the cabinet 2, by means of a controllable electric heater 15.

A microscope 16, viewing into the chamber 2 through a heated window 17 is provided with an independent tungsten filament spot light (not shown) with a suitable heat filter.

The cell 14 can be supplied with low temperature liquid nitrogen from the storage vessel 8 by means of an independent feed line (not shown). The vessel 8 is arranged so that it may be refilled or topped-up without disturbing the functioning of the equipment.

WHAT WE CLAIM IS:—

1. A cryostat assembly comprising a thermally insulated cryostat chamber and a thermally insulated container in non-closable free-flow fluid communication therewith, the container being adapted to contain a liquified non-oxidising gas for cooling the chamber to a predetermined temperature, the supply of liquified gas through the fluid communication to the chamber being controlled by control means including an electric heating means positioned inside the thermally insulated container, the control means controlling the supply of current to said electric heating means so as to supply heat to the liquified gas in said container to thereby vapourise controllable quantities of said liquified gas to pressurise the container, the pressure so generated forcing a controlled quantity of the liquified gas through the fluid communication into said chamber, the arrangement being such that the said controlled quantity of gas evaporates in said chamber to cool the chamber.

2. A cryostat assembly as claimed in Claim 1, wherein the thermally insulated container is external of the chamber and communicates with the interior of the chamber by way of a tube through which the liquified gas can flow.

3. A cryostat assembly as claimed in Claim 1 or 2 wherein an additional standby mechanical refrigeration system is provided to maintain the interior of the chamber at a standby temperature higher than said predetermined temperature.

4. A cryostat assembly as claimed in any preceding claim wherein the chamber contains a microtome and a vacuum probe is provided to manipulate the cut sections of material provided by said microtomes.

5. A cryostat assembly as claimed in Claim 4 wherein said vacuum probe is connected to a micro manipulator which

has manually operable controls for controlling movement of the probe in three mutually perpendicular planes, the controls being provided externally of the chamber.

6. A cryostat assembly as claimed in Claim 4 or 5 wherein further temperature control means are provided for controlling the temperature of a knife of said microtome independently of the temperature inside said chamber.

7. A cryostat assembly as claimed in Claim 6 wherein said further temperature control means includes an electric heater.

8. A cryostat assembly as claimed in any one of the preceding Claims wherein a thermally insulated light-tight compartment is provided within said chamber.

9. A cryostat assembly as claimed in Claim 9 wherein means are provided for supplying controllable quantities of liquified gas to said compartment.

10. A cryostat assembly substantially as described herein with reference to the accompanying drawings.

JENSEN & SON,
Agents for the Applicants
8, Fulwood Place
High Holborn,
London WC1V 6HG.
Chartered Patent Agents.

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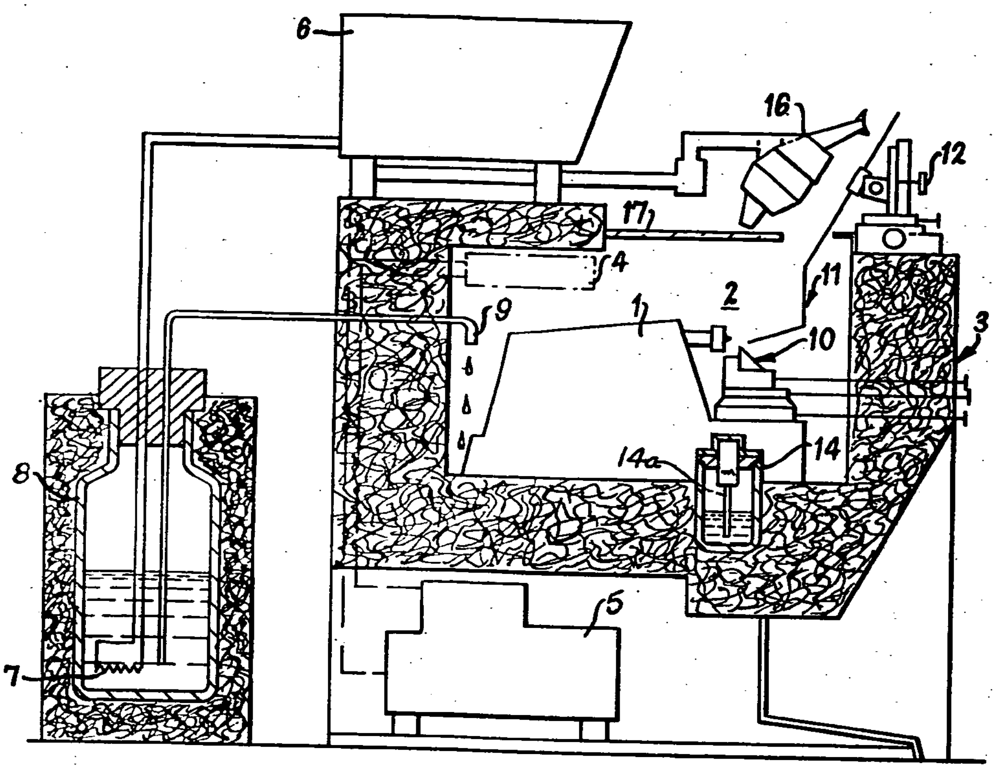

Fig. 1

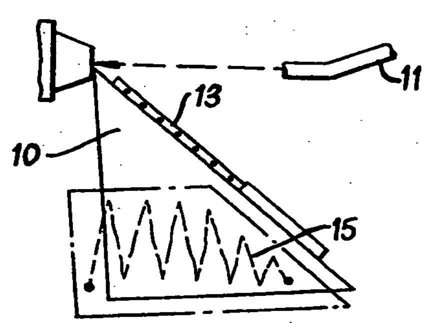

Fig. 2